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Formation of oxygenated α,β-unsaturated aldehydes and other toxic compounds in sunflower oil oxidation at room temperature in closed receptacles

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ABSTRACT

The study of the volatile components presents in the headspace of 27 sunflower oil samples, stored over different periods of time, at room temperature in closed receptacles, in presence of limited amounts of air, has been carried out by solid phase microextraction (SPME) followed by gas chromatography/mass spectrometry (GC/MS). The composition of the headspace of the studied samples is highly varied, ranging from the characteristic of non-oxidized oils to that of oils with a high oxidation level. Among the detected compounds are alkanals, (*E*)-2-alkenals, 2,4-alkadienals, ketones, acids, esters, alcohols, as well as aliphatic and aromatic hydrocarbons. In samples having a certain oxidation degree it is worth noting the presence of a significant number of genotoxic and cytotoxic oxygenated α,β−unsaturated aldehydes (OαβUAs), such as 4-hydroxy-2-nonenal, 4-oxo-2-nonenal or 4,5-epoxy-2-decenal, which have been considered responsible for various degenerative diseases which are widespread nowadays; some of these compounds are described in this manuscript for the first time as coming from edible oil oxidation at room temperature. In addition, it has been found the occurrence of polycyclic aromatic hydrocarbons (PAHs) in concentrations in line with the oxidation level of the sample.

1. Introduction

It is well known that the oxidation process of edible oils is a complex subject of great interest given its economical and technological repercussions, as well as to its direct effects on consumer health. Recent studies by ^{1}H Nuclear Magnetic Resonance of the liquid phase of edible oils submitted to 70 °C with aeration have shown that under these oxidative conditions some oxygenated α,β-unsaturated aldehydes (OαβUAs), such as 4-hydroxy-, 4-hydroperoxy-, and 4,5-epoxy-(*E*)-2-alkenals, are formed in different proportions, depending on the oil nature (Guillén & Ruiz, 2004, 2005a, 2005b, 2005c; Guillén, Cabo, Ibargoitia, & Ruiz, 2005). Likewise, previous studies have reported that some of these oxygenated α,β-unsaturated aldehydes, such as 4-hydroxy-(*E*)-2-nonenal, can also be formed endogenously in the oxidative stress of cells and tissues (Esterbauer, Schaur, & Zollner, 1991). They have been linked to degenerative diseases such as cancer, Alzheimer's or Parkinson's, among others (Esterbauer et al., 1991; Zarkovic, 2003) and have shown considerable reactivity. Some of them have also been detected in foods (Guillén & Goicoechea, 2008).

The mechanisms of the oxidation processes of edible oils largely depend on the oxidative conditions. For this reason, it could be thought that either the nature or the proportions of the volatile compounds produced in the oxidation process of edible oils at room temperature, in closed receptacles, could be different from those produced at higher temperatures (Guillén et al., 2005).

As the oxidation process of edible oils at low temperature is very slow, there are few studies taking place under these conditions. This paper studies the headspace composition of 27 samples of sunflower oil which have been stored in closed bottles at room temperature, in the presence of limited amounts of air for different periods of time, thus being at different oxidation stages; the study is carried out by means of solid phase microextraction (SPME) followed by gas chromatography/mass spectrometry (GC/MS). The oxidative status of these 27 samples was studied before by means of Fourier Transform Infrared Spectroscopy (FTIR) and Proton Nuclear Magnetic Resonance (^{1}H NMR) (Guillén & Goicoechea, 2007).

To the best of our knowledge the headspace composition of sunflower oil oxidized in closed receptacles at room temperature in presence of reduced concentrations of air has not been studied before. For this reason, this study may provide new knowledge about this oxidation process; it should be taken into account that although many volatile compounds have been identified as coming from oil oxidation processes, many others still remain to be identified.

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2. Materials and methods

2.1. Samples, oxidation conditions and standards

The study was carried out on 27 sunflower oil samples, acquired from local supermarkets throughout ten years, and stored in non-permeable containers, at room temperature during different periods of time, with different air–oil volume ratios and different air–oil contact surfaces; the containers were not reopened once stored. In this group of samples there are sunflower oils of the same brand and batch stored with different air–oil volume ratios, oils of the same brand but a different batch acquired one or more years later, and also oils of different brands.

The compositions of these oils, when they were acquired, were in agreement with the legal requirements demanded by the European Union for edible sunflower oils, ranging their acyl group proportions between 12% and 13% in weight of saturated acyl groups, between 22% and 27% in weight of oleic acyl groups, and between 60% and 63% in weight of linoleic acyl groups; it should be taken into account that nowadays the sunflower oils present in the supermarkets, to be acquired by consumers, are made of mixtures of oils coming from very different places of the world.

Under these storage conditions the several sunflower oil samples reached very varied oxidation levels, which were evaluated from the study of their liquid phase by Fourier transform infrared spectroscopy and by ^{1}H nuclear magnetic resonance (Guillén & Goicoechea, 2007). The samples were named from S1 to S27, being in successively higher oxidation stages, and Table 1 gives the conditions of storage and the relative proportions of hydroperoxides and of aldehydes determined from ^{1}H nuclear magnetic resonance spectral data (Guillén & Goicoechea, 2007).

Compounds, such as 4-oxo-(*E*)-2-nonenal (CAS 103560-62-9), 4-hydroxy-(*E*)-2-nonenal (CAS 75899-68-2), 4-hydroperoxy-(*E*)-2-nonenal (CAS 83920-83-6) and 4,5-epoxy-(*E*)-2-decenal (CAS 134454-31-2) acquired from Cayman Chemical (Ann Arbor, MI, USA), and the other compounds asterisked in the different tables acquired from Aldrich (Milwaukee, WI, USA), were used as standard compounds for identification purposes.

Table 1
Some of the oxidation conditions during storage, such as storage time (ST), air–oil contact surface (CS), air volume (AV), oil volume (OV), and air–oil volume ratio (AOVR), together with the relative molar proportions of hydroperoxides (HY) and of aldehydes (AL) present in the sunflower oil samples (Guillén & Goicoechea, 2007)

Sample	ST (months)	CS (cm^2)	AV (cm^3)	OV (cm^3)	AOVR	HY	AL
S1	40	11.3	4.1	828.6	0.005	0.065	0.000
S2	63	38.5	100.5	769.7	0.131	0.134	0.000
S3	90	36.3	118.8	1700.3	0.070	0.257	0.000
S4	4	15.2	10.9	860.1	0.013	0.096	0.000
S5	4	15.2	10.9	898.6	0.012	0.075	0.000
S6	4	15.2	10.9	898.6	0.012	0.075	0.000
S7	63	32.2	45.3	729.6	0.062	0.270	0.000
S8	64	38.5	254.5	615.7	0.413	0.316	0.000
S9	40	15.2	10.9	840.8	0.013	0.152	0.000
S10	53	34.2	34.2	581.6	0.059	0.195	0.000
S11	4	15.2	10.9	860.1	0.013	0.250	0.000
S12	53	38.5	119.8	731.2	0.164	0.273	0.000
S13	112	69.4	328.7	1492.4	0.220	0.372	0.000
S14	6	15.2	10.9	860.1	0.013	0.313	0.000
S15	71	38.5	312.2	596.5	0.523	0.296	0.000
S16	105	38.5	466.1	423.3	1.101	0.697	0.000
S17	90	38.5	697.0	192.4	3.622	0.782	0.170
S18	106	12.6	12.6	62.8	0.200	1.391	0.226
S19	77	38.5	754.8	134.7	5.604	1.854	0.287
S20	101	12.6	6.3	69.2	0.091	2.802	0.338
S21	112	12.6	50.3	25.1	2.000	2.446	0.487
S22	112	12.6	37.7	37.7	1.000	2.044	0.779
S23	106	12.6	37.7	37.7	1.000	2.091	0.751
S24	112	12.6	56.5	18.8	3.000	1.138	0.694
S25	112	12.6	56.5	18.8	3.000	1.013	0.695
S26	112	12.6	56.5	18.8	3.000	0.965	0.762
S27	106	12.6	62.8	12.6	5.000	0.322	1.489

2.2. Extraction of the oil headspace components by SPME

Vials containing 1 g of oil were introduced into a water bath maintained at 50 °C. After a period of sample equilibration (15 min), a fiber coated of DVB/CAR/PDMS (Divinylbenzene/Carboxen/Polydimethylsiloxane, 50/30 μm film thickness), acquired from Supelco, was inserted into the headspace of the sample and was maintained for 60 min. The selection of the fiber type and of the extraction operating conditions was previously studied in our laboratory.

2.3. Gas chromatography/mass spectrometry study

The fibre was desorbed for 10 min in the injection port of a Hewlett-Packard gas chromatograph model HP 6890 Series II, equipped with a Mass Selective Detector 5973 and a Hewlett-Packard Vectra XM Series 4 computer operating with the ChemStation program. The column used was a fused-silica capillary column (60 m long × 0.25 mm inner diameter × 0.25 μm film thickness; from Hewlett-Packard), coated with a non-polar stationary phase (HP-5MS, 5% phenyl methyl siloxane). The operation conditions were the following: the oven temperature was set initially at 50 °C (5 min hold), increased to 280 °C at 4 °C/min (2 min hold); the temperatures of the ion source and the quadrupole mass analyzer were kept at 230 and 150 °C, respectively; Helium was used as carrier gas at a pressure of 16.5 psi; injector and detector temperatures were held at 220 and 280 °C, respectively; splitless mode was used for injection with a purge time of 1.5 min. Mass spectra were recorded at ionization energy of 70 eV; the data acquisition mode employed was Scan. After the first desorption, the fiber was routinely submitted to desorption conditions for a second time for its cleaning up and at the same time to determine if the first process was complete.

Many components were identified by using standards. So, asterisked compounds in the several tables were acquired commercially and used as standards for identification. Many other components were only tentatively identified. In this latter case, retention times, together with mass spectra, and matching with mass spectra of a commercial library higher than 85%, were taken as identification criteria (Wiley 275.L, Mass Spectral Database, Rev.D.01.00, June 2000) as in previous studies (Guillén et al., 2005). The semi quantification of the components was based on the area counts of the base peak of the mass spectrum of each compound divided by 10^6. The base peak is the most intense peak (intensity 100%) in the mass spectrum of a compound. Although the chromatographic response factor of each compound is different, the area counts so determined are useful for comparative purposes of each compound in different samples. The results were obtained as average values of two determinations.

3. Results and discussion

It is well known that in their oxidation edible oils first form primary oxidation compounds or hydroperoxides, whose degradation generates secondary oxidation compounds; among these there are, in addition to polymers, oligomers and monomers of modified triglycerides, volatile compounds. This latter group is constituted among other compounds by acids, alcohols, esters, hydrocarbons, and furan and carbonylic derivatives. However, the proportions and nature of the compounds generated in each oil oxidation process depend on the oil composition and on the oxidative conditions

under which the process takes place, and although many of these compounds have been identified for a long time, many other still remain unidentified.

The SPME technique followed by GC/MS has been shown to be a suitable method for evaluating the oxidation degree of edible oils. This has been demonstrated by comparison with classical parameters, such as peroxide value, UV spectrophotometric absorbance, and loss of unsaturated fatty acids (Vichi, Pizzale, Conte, Buxaderas, & Lopez-Tamames, 2003) and also by comparison with spectroscopical FTIR and ^{1}H NMR data (Guillén et al., 2005).

As it was expected, when comparing the results obtained from the study of the oxidative status of the liquid phase above mentioned, carried out with spectroscopic techniques (Guillén & Goicoechea, 2007) (see Table 1), with the chromatograms of the headspace of the same samples, it was observed that the composition of this latter, in number and concentration of compounds, was in line with oxidation level of the samples. Although the 27 samples were studied, for practical reasons, Fig. 1 gives the gas chromatograms of the headspace components of only five sunflower oil samples, named S6, S15, S17, S18 and S22, selected among all those studied as being representative of samples with different oxidation level. For the same reason, the results and discussion are referred to these five samples. These samples are coming from different brands; however, there were no significant differences in the composition of these oils prior to storage. It can be observed that the headspace of sample S6 is very poor in components, corresponding to a non-oxidized sample, and the number of components and their concentrations are progressively higher in the headspace of the samples S15, S17, S18 and S22, which have increasing oxidation levels. In the headspace of sample S15 only those peaks of compounds eluting before 21 min show certain intensity; however, in the headspace of sample S17, in addition to these, the peaks of other compounds eluting after 21 min are clearly visible. And finally, the headspaces of samples S18 and S22, as Fig. 1 shows, are the richest in number and in concentration of components.

In order to summarize the nature and proportions of the volatile compounds found in the headspace of the 27 sunflower oil samples, Tables 2–5 show those found in the headspace of the S6, S15, S17, S18 and S22 samples.

It can be observed in Table 2 that in the headspace of the S6 sunflower oil sample the only acid detected is acetic. However, as the sample oxidation level increases a corresponding increase

Table 2
Acids, alcohols and esters detected in the headspace of S6, S15, S17, S18 and S22 sunflower oil and their abundances, expressed as area counts of their mass spectra base peak (Bp) divided by 10^6, obtained as average of two determinations

Compound (molecular weight)[a]	Bp	S6	S15	S17	S18	S22
Acids		*0.00*	*38.55*	*137.39*	*210.88*	*341.66*
Formic acid (46)	46	0.00	10.67	17.38	14.16	24.57
Acetic acid (60)*	43	0.58	3.52	17.64	22.53	28.38
Propanoic acid (74)*	74	0.00	0.07	0.16	0.34	0.51
Butanoic acid (88)*	60	0.00	0.27	0.38	0.85	1.47
Pentanoic acid (88)*	60	0.00	3.28	8.98	12.62	21.29
Hexanoic acid (116)*	60	0.00	20.31	91.17	152.76	253.81
Heptanoic acid (130)*	60	0.00	0.23	1.13	3.70	5.06
Octanoic acid (144)*	60	0.00	0.13	0.37	3.10	5.00
Nonanoic acid (158)*	60	0.00	0.07	0.18	0.79	1.54
Decanoic acid (172)	73	0.00	0.00	0.00	0.03	0.03
Alcohols		*0.09*	*4.47*	*26.35*	*34.88*	*48.31*
1-Butanol (74)	56	0.00	0.00	0.07	0.10	0.14
1-Pentel-3-ol (86) (or isomer)	57	0.00	0.00	0.00	0.22	0.34
1-Pentanol (88)*	42	0.00	0.21	1.42	1.77	3.61
Methylcyclopentanol (100) (or isomer)	57	0.00	0.00	0.03	0.03	0.07
1-Hexanol (102)*	56	0.00	0.38	1.69	1.70	2.47
1-Hepten-3-ol (114) (or isomer)	57	0.00	0.00	0.37	0.21	1.04
Cyclohexanol (100)	57	0.00	0.05	0.27	0.37	0.24
1-Octen-3-ol (128)*	57	0.09	3.83	21.61	29.28	36.64
Cyclooctanol (128) (or isomer)	57	0.00	0.00	0.73	0.78	1.86
4-Ethylcyclohexanol (128) (or isomer)	81	0.00	0.00	0.12	0.33	1.71
4-Nonanol (144)	55	0.00	tr	0.04	0.09	0.19
Esters		*4.99*	*11.62*	*4.29*	*3.43*	*3.84*
Ethyl acetate(88)*	43	4.99	11.62	4.10	2.97	2.62
Pentyl methanoate (116) (or isomer)	42	0.00	0.00	0.17	0.26	0.86
Pentyl hexanoate (186) (or isomer)	70	0.00	0.00	0.02	0.12	0.17
Dibutyl-2-butenedioate (228)	99	0.00	0.00	0.00	0.08	0.19

[a] Asterisked compounds were acquired commercially and used as standards for identification purposes; tr traces.

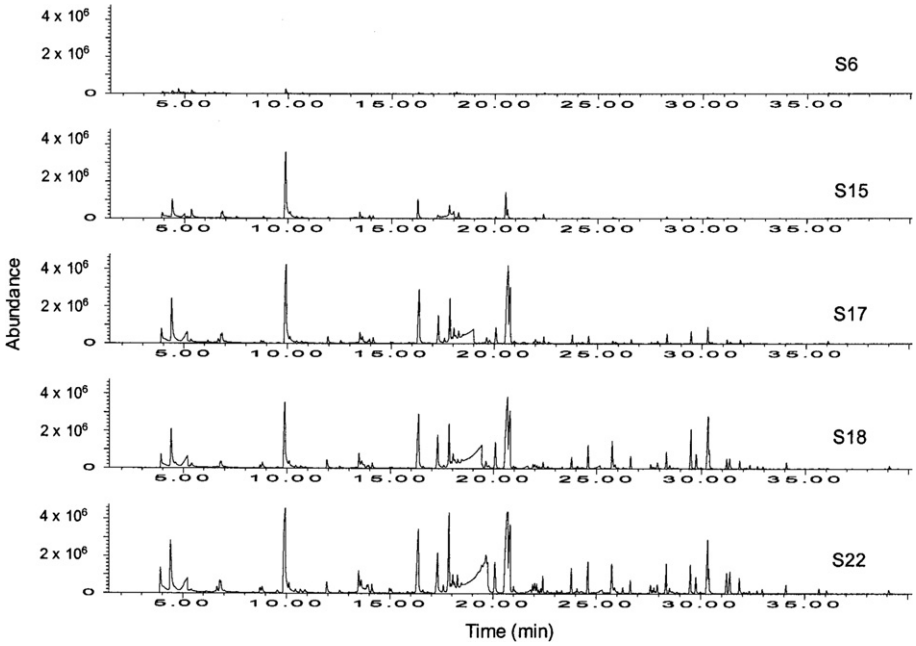

Fig. 1. Chromatograms of the headspace components extracted by SPME from the sunflower oil samples S6, S15, S17, S18 and S22, selected among the 27 samples studied, as being representative of the different oxidation levels reached during storage.

in the number and concentration of the acids is observed The main detected acids are hexanoic, acetic and formic acids, followed by pentanoic, heptanoic and octanoic acids; propanoic, butanoic and nonanoic acids have been also detected in all samples, but decanoic acid is only present in the most oxidized samples (see S18 and S22).

As might be expected, in addition to acids, alcohols are also present in these samples. In the headspace of the non-oxidized sunflower oil S6 only 1-octen-3-ol was detected, and it increases in line with the oxidation level of the sample. However, the headspace of the samples from S15 to S22, as Table 2 shows, contains some other alcohols, such as 1-pentanol and 1-hexanol. The headspace of samples with a certain oxidation level (S17, S18, and S22) contains other alcohols but in very low proportions.

Esters also occur in these samples. As indicated in Table 2, ethylacetate was detected in the headspace of all samples kept at room temperature, but its occurrence seems to be independent of the oxidation degree of the sample. However, the remaining esters in Table 2 were only present in oxidized samples in low proportions, but growing with the oxidation degree of the sample.

In addition to the above mentioned compounds, a great number of hydrocarbons were present in the headspace of the samples and they have been grouped in Table 3 as saturated, monounsaturated, diunsaturated and cyclic hydrocarbons; terpenes, sesquiterpenes and related compounds; monoaromatic hydrocarbons; and polyaromatic hydrocarbons.

It is well known that non-oxidized oil samples can have small proportions of hydrocarbons, mainly linear hydrocarbons, as is observable in sample S6 (see Table 3). However, the occurrence and concentration of saturated, unsaturated or cyclic hydrocarbons in the headspace of these oil samples is higher as higher the oxidation degree of the sample is. In spite of the large number of compounds of this group, only some of them can be considered to be in significant proportions, being the main the aliphatic saturated hydrocarbons that have an uneven number of carbon atoms, such as nonane, heptane and pentane. Furthermore, monounsaturated hydrocarbons with from 6 to 16 carbon atoms have also been detected, as well as some hydrocarbons tentatively identified as diunsaturated having from 8 to 11 carbon atoms.

The presence of terpenes, sesquiterpenes and of triterpenes is common in non-oxidized vegetable oils. These compounds are formed in the normal metabolism of many plants. Some of them have been detected in the headspace of the sunflower oil samples here studied as can be observed in Table 3. The concentration of these compounds apparently is not related to the oxidation degree of the sample. However, the headspace of the most oxidized sample (S22) contains a larger number of these compounds, in slightly higher concentrations, than the headspace of less oxidized samples; for this reason, the possible formation of these latter compounds cannot be discarded, in the oxidation produced at room temperature in presence of a limited amount of air.

These compounds could have been formed as a consequence of termination reactions of alkyl free radicals formed in the process, without significant proportions of oxygen.

When it comes to benzene derivatives, the presence in non-oxidized edible oils of small proportions of methyl or dimethyl benzene derivatives has been reported previously (Vichi, Pizzale, Conte, Buxaderas, & Lopez-Tamames, 2005) and this is in agreement with the presence of some alkyl benzene derivatives in very low proportions in the non-oxidized sample S6. However, it is clearly observable in Table 3 that both, the number of compounds of this nature in the headspaces of these samples, and also their concentrations, rise with the oxidation degree of the sample. This is very clear in the case of toluene and also in the case of other alkyl benzene derivatives. These results could suggest the existence of a close relationship between the oxidation degree and the presence and concentration of monoaromatic hydrocarbons in the sample. This in turn suggests that the oxidation of sunflower oil, having important proportions of diunsaturated acyl groups, produced at room temperature in closed receptacles under limited amounts of oxygen, can lead to the formation of alkyl benzene derivatives. To the best of our knowledge this is the first time that the formation of alkylbenzene derivatives has been associated with the oxidation of oils, containing diunsaturated acyl groups, in presence of limited amounts of oxygen. The formation of these compounds could be considered as coming from alkyl unsaturated free radicals, produced in the oxidation, which could evolve to form molecules of alkyl benzene derivatives in an environment with limited content in oxygen. Some authors have also detected the presence of alkylbenzenes in used frying soyabean or cottonseed oils, although their origin has not been discussed (Takeoka, Perrino, & Buttery, 1996).

As previously mentioned, the presence of toxic benzene derivatives (Janik-Spiechowicz & Wyszynska, 1999) in edible oils of several vegetable sources has been reported by some authors, and the origin of these compounds has been attributed to the contamination of the working area and storage, or to the level of these compounds in the fruits or seeds at the crop period; likewise, the increase of the concentration of these contaminants in fruit during storage has also been attributed to environmental contamination (Biedermann, Grob, & Morchio, 1995; Morchio, Spadone, & Bracco, 1994). The possibility of formation of these compounds in oil seeds or in olives in the plant or out of the plant as consequence of stress oxidative processes cannot be discarded and in fact some of these authors have found levels of alkyl derivatives in oily fruits which were higher than those expected from environmental contamination (Biedermann, Grob, & Morchio, 1996). In this context, some authors have pointed out that styrene is a product of metabolism of olives, because its concentration strongly increased during storage of crushed olives at ambient temperature (Biedermann et al., 1995).

In addition to the above mentioned hydrocarbons, some Polycyclic Aromatic Hydrocarbons (PAHs) were also detected, as Table 3 shows. The presence of compounds of this family in edible oils of very different nature is well known and has been shown for a long time (Guillén, Sopelana, & Palencia, 2004); for this reason, due to their toxicity, legal limits for the occurrence of some of them in oils have been recently introduced in the European Union and in some European countries (Commission Regulation EC No. 208/2005, 2005). The PAHs detected in the headspace of some of the samples were naphthalene, their two methyl derivatives, some of their dimethyl derivatives, especially 1,6-dimethylnaphthalene, biphenyl and 9H-fluorene. Although the occurrence of compounds of this nature is expected in oils, as above commented, it is noteworthy that the presence and concentration of these compounds in the headspace of the samples goes up with the oxidation degree of the sample, and that this effect is noticeable in naphthalene, methyl-naphthalenes, 1,6-dimethyl-naphthalene and 9H-fluorene. In this context, it should be mentioned that although the laws about limits of PAHs in oils make reference to heavy compounds, naphthalene has been recently considered as "*possibly carcinogenic to humans*" (group 2b) by the International Agency for Research on Cancer (IARC, 2002). The study of the liquid phase of these same samples by ^{1}H NMR revealed that among the hydroperoxides and hydroxides formed, by contrast with those formed at higher temperatures, the (*Z,E*) conjugated dienic systems predominate versus the (*E,E*) (Guillén & Goicoechea, 2007). The presence of significant concentrations of unsaturated free radicals having (*Z,E*) isomerism could favour the formation of cyclic compounds. This cyclation of unsaturated free radicals could also be favoured by the increase in the pressure in the closed system, provoked by the growing presence of volatile compounds generated in the oxidation.

Table 3
Hydrocarbons and some related compounds detected in the headspace of S6, S15, S17, S18 and S22 sunflower oil samples and their abundances expressed as area counts of their mass spectra base peak (Bp) divided by 10^6, obtained as average of two determinations

Compound (molecular weight)[a]	Bp	S6	S15	S17	S18	S22
Saturated hydrocarbons		*5.15*	*16.20*	*41.75*	*43.61*	*72.58*
Butane (58)	43	0.06	0.19	0.58	0.27	0.89
Pentane (72)*	43	3.95	10.76	31.53	31.39	42.27
Hexane (86)*	57	0.09	0.13	0.25	0.31	0.57
Heptane (100)*	56	0.15	0.90	1.37	0.96	1.63
Octane (114)	85	0.49	3.09	3.73	2.60	3.74
Trimethylhexane (128)	43	0.00	0.00	0.00	0.20	0.20
4-Methyloctane (128)	43	0.00	0.00	0.00	0.15	0.13
Nonane (128)	43	0.01	0.02	0.65	1.00	2.24
3-Methylnonane (142)	57	0.16	0.02	0.00	0.00	0.00
Decane (142)*	142	0.04	0.01	0.01	0.02	0.02
Methyldecane (156) (or isomer)	57	0.00	0.00	0.00	0.08	0.12
Branched hydrocarbon (140)	55	0.00	0.00	0.00	0.40	1.71
Branched hydrocarbon (140)	69	0.05	0.07	0.41	0.65	1.94
Undecane (156)	57	0.02	0.14	0.60	0.68	1.08
Dimethyldecane (170) (or isomer)	57	0.00	0.00	0.00	0.00	0.15
Dodecane (170)*	57	0.14	0.11	0.13	0.43	0.65
Dodecane (170) (or isomer)	57	0.00	0.00	0.00	0.05	0.07
Tridecane (184)*	57	0.00	0.02	0.07	0.26	0.45
Tetradecane (198)*	57	0.00	0.04	0.11	0.70	1.34
Methyltetradecane (212)	57	0.00	0.00	0.00	0.05	0.09
Pentadecane (212)*	57	0.00	0.00	0.00	0.20	0.28
Monounsaturated hydrocarbons		*0.23*	*2.06*	*5.06*	*4.00*	*9.63*
1-Hexene (84)*	56	0.00	0.00	0.46	0.18	0.61
1-Heptene (98)*	56	0.04	0.15	1.66	0.46	2.42
2-Heptene (98)	56	0.00	0.11	0.44	0.25	0.66
Heptene (98) (or isomer)	56	0.00	0.00	0.14	0.06	0.22
Octene (112) (or isomer)	55	0.05	0.23	0.29	0.30	0.89
Octene (112) (or isomer)	55	0.08	1.15	1.13	1.35	2.06
Octene (112) (or isomer)	55	0.06	0.41	0.46	0.56	0.93
Nonene (126) (or isomer)	55	0.00	0.00	0.42	0.29	0.64
Nonene (126) (or isomer)	55	0.00	0.00	0.00	0.00	0.09
2-Nonene (126) (or isomer)	126	0.00	0.01	0.02	0.02	0.12
Nonene isomer (126)	55	0.00	0.00	0.04	0.03	0.18
1-Tetradecene (196)	55	0.00	0.00	0.00	0.34	0.47
Methyleneundecane (168) (or isomer)	70	0.00	0.00	0.00	0.09	0.21
1-Hexadecene (224)*	41	0.00	0.00	0.00	0.07	0.13
Diunsaturated hydrocarbons		*0.00*	*0.02*	*1.24*	*1.29*	*3.93*
Octadiene (110) (or isomer)	110	0.00	0.04	0.09	0.07	0.22
Nonadiene (124) (or isomer)	54	0.00	0.04	0.04	0.45	1.83
Nonadiene (124) (or isomer)	54	0.00	0.02	0.26	0.29	0.82
Nonadiene (124) (or isomer)	124	0.00	0.01	0.04	0.02	0.14
Ethylmethylhexadiene (124) (or isomer)	67	0.00	0.51	0.34	0.25	0.60
Undecadiene (152) (or isomer)	67	0.00	0.00	0.07	0.21	0.32
Cyclic hydrocarbons		*0.22*	*1.04*	*1.24*	*0.57*	*1.43*
Cyclohexane (84)*	84	0.22	0.09	0.87	0.16	0.22
Cycloheptene (96) (or isomer)	56	0.00	0.00	0.02	0.02	0.09
Methylcyclohexane (98) (or isomer)	83	0.00	0.91	0.00	0.00	0.00
Methylpentylcyclopropane (126) (or isomer)	56	0.00	0.00	0.28	0.28	0.90
Propylcyclohexane (126) (or isomer)	83	0.00	0.02	0.03	0.04	0.08
Butylcyclopentane (126) (or isomer)	69	0,00	0,02	0,04	0,04	0,08
Octylcyclohexane (196) (or isomer)	83	0.00	0.00	0.00	0.03	0.06
Terpenes. sesquiterpenes and related compounds		*0.21*	*0.31*	*0.42*	*0.88*	*1.22*
α-Pinene (136) (or isomer)	93	0.04	0.06	0.07	0.19	0.25
Limonene (136)*	68	0.03	0.06	0.12	0.67	0.89
α-Campholene aldehyde (152)	108	0.14	0.07	0.02	0.00	0.00
Sesquiterpene (204)	204	0.00	0.00	0.00	0.00	tr
α-Ylangene (204) (or isomer)	204	0.00	0.00	0.00	0.00	tr
Junipene (204)	161	0.00	0.02	0.05	0.02	0.07
Calarene (204)	161	0.00	0.08	0.13	0.00	0.00
(*E*)-Farnesene (204) (or isomer)	204	0.00	0.00	0.00	0.00	tr
Aristolen (204) (or isomer)	204	0.00	0.00	0.00	0.00	tr
Triterpene (272)	272	tr	tr	tr	0.00	tr
Kaur-16-ene (272)	257	0.00	0.02	0.03	0.00	0.01
Monoaromatic hydrocarbons		*0.36*	*2.44*	*2.65*	*8.79*	*8.96*
Benzene (78)*	78	0.00	0.00	0.06	0.00	0.17
Methylbenzene (toluene) (92)*	91	0.16	2.06	2.10	7.06	7.33
Dimethylbenzene (106)	91	0.03	0.04	0.04	0.07	0.07
Dimethylbenzene (106)	91	0.11	0.12	0.12	0.22	0.22
Dimethylbenzene (106)	91	0.02	0.02	0.02	0.05	0.05
Methylethylbenzene (120) (or isomer)	120	0.00	0.00	0.01	0.02	0.03
Trimethylbenzene (120)	105	0.01	0.01	0.03	0.08	0.11
1-Methyl-2-(1-methylethyl)-benzene (134) (or isomer)	119	0.03	0.08	0.08	0.87	0.38
1-Methyl-4-propylbenzene (134) (or isomer)	105	0.00	0.00	0.00	0.03	0.04
(1,1-Dimethylethyl)-benzene (134) (or isomer)	119	0.00	0.00	0.01	0.03	0.03
(1,1-Dimethylethyl)-benzene (134) (or isomer)	119	0.00	0.00	0.01	0.03	0.05
(2-Methyl-2-propenyl)-benzene (132) (or isomer)	117	tr	0.02	0.04	0.07	0.12
(2-Methyl-2-propenyl)-benzene (132) (or isomer)	117	tr	0.04	0.06	0.14	0.18
(2-Methyl-2-propenyl)-benzene (132) (or isomer)	117	0.00	0.04	0.07	0.10	0.16
Octylbenzene (190)	91	0.00	0.00	0.00	0.02	0.02
Polyaromatic hydrocarbons		*0.01*	*2.48*	*4.92*	*49.90*	*58.92*
Naphthalene (128)*	128	0.01	1.67	3.45	25.72	29.18
2-Methylnaphthalene (142)*	142	0.00	0.32	0.47	8.79	10.17
1-Methylnaphthalene (142)*	142	0.00	0.43	0.79	10.42	13.62
1,1́-Biphenyl (154)*	154	0.00	0.00	0.01	0.05	0.07
Dimethylnaphthalene (156)	156	0.00	0.00	0.00	0.03	0.03
Dimethylnaphthalene (156)	156	0.00	0.00	0.00	0.04	0.06
1,6-Dimethylnaphthalene (156)*	156	0.00	0.06	0.15	3.05	3.81
Dimethylnaphthalene (156)	156	0.00	0.00	0.00	0.01	0.02
Dimethylnaphthalene (156)	156	0.00	0.00	0.00	0.00	0.01
2-Methyl-1,1-biphenyl (168)	168	0.00	0.00	0.00	0.01	0.02
9H-Fluorene (166)*	166	0.00	0.00	0.05	1.78	1.93

[a] Asterisked compounds were acquired commercially and used as standards for identification purposes; tr traces.

Accordingly with these results, it has also been proved the formation of PAHs in soil under oxygen deficient conditions (Thiele & Brummer, 2002).

As it has been commented above, many of the 27 samples studied are of different brand and/or batch and, for this reason, it could be thought that the current difference in PAHs concentration in their headspaces could be due to the different original concentration of these compounds in the several samples before their oxidation. However, some of the other studied samples are coming from the same brand, batch and bottle, and initially contained the same concentration of PAHs, and after storage their headspaces have different concentration of these toxic compounds. These results coming from the oil gas phase were confirmed by the determination of the PAHs concentration in the liquid matrix of two sunflower oil samples stored under different conditions but coming from the same original oil (Guillén, Goicoechea, Palencia, & Cosmes, 2008).

It is also well known that carbonyl derivatives are among the main compounds generated in the lipid oxidation. Table 4 shows the compounds of this nature, among which are ketones grouped in linear saturated and unsaturated ketones, cyclic ketones, and diketones. As can be observed in this table the headspace of non-oxidized sample S6 is free of ketones, but as expected, the presence and concentration of ketones increases with the oxidation level of the samples studied. Among the detected ketones, 2-heptanone and 3-nonen-2-one are significant, followed by 3-octen-2-one and 2-nonanone.

Aldehydes constitute the most numerous group of carbonyl derivatives formed in any oil oxidation process. Among the aldehydes detected in this study, shown in Table 4, are alkanals, alkenals, alkadienals and alkatrienals, and aromatic aldehydes. Alkanals from

Table 4
Carbonyl derivatives detected in the headspace of S6, S15, S17, S18 and S22 sunflower oil samples and their abundances expressed as area counts of their mass spectra base peak (Bp) divided by 10^6, obtained as average of two determinations

Compound (molecular weight)[a]	Bp	S6	S15	S17	S18	S22
Saturated linear ketones		*0.00*	*5.36*	*11.57*	*16.17*	*28.52*
2-Butanone (72)*	72	0.00	0.00	0.17	0.09	0.29
2-Pentanone (86)*	43	0.00	0.18	0.18	0.35	0.58
2-Hexanone (100)*	100	0.00	0.07	0.09	0.12	0.12
3-Heptanone (114)	114	0.00	0.00	0.01	0.01	0.03
2-Heptanone (114)*	43	0.00	4.49	7.54	10.13	15.74
3-Octanone (128)	43	0.00	0.13	1.10	0.98	2.73
2-Octanone (128)*	43	0.00	0.32	0.79	1.16	2.15
4-Octanone (128)	57	0.00	0.00	0.59	0.64	0.90
2-Nonanone (142)*	43	0.00	0.20	0.89	1.63	3.83
2-Decanone (156)*	58	0.00	0.04	0.08	0.75	1.33
6-Undecanone (170)	71	0.00	0.02	0.07	0.22	0.35
2-Undecanone (170)	58	0.00	0.00	0.00	0.03	0.05
6-Dodecanone (184)	43	0.00	0.00	0.08	0.19	0.43
2-Dodecanone (184)	58	0.00	0.00	0.00	0.01	0.02
Unsaturated linear ketones		*0.00*	*0.56*	*5.33*	*6.25*	*13.59*
1-Hepten-3-one (112)	55	0.00	0.00	0.30	0.13	0.52
1-Octen-3-one (126) (or isomer)	126	0.00	0.00	0.00	0.00	0.01
3-Octen-2-one(126)	55	0.00	0.31	1.62	1.66	2.82
2-Nonen-4-one (140)	69	0.00	0.00	0.11	0.08	0.27
3-Nonen-2-one (140)	55	0.00	0.25	3.30	4.38	9.97
Cyclic ketones		*0.00*	*0.01*	*0.02*	*0.04*	*0.23*
Methylcyclopentanone (98) (or isomer)	98	0.00	0.01	0.02	0.03	0.07
Methylcyclopentanone (98) (or isomer)	98	0.00	tr	tr	0.01	0.03
Methylcycloheptenone (124) (or isomer)	67	0.00	0.00	0.00	0.00	0.13
Diketones		*0.00*	*0.07*	*0.21*	*0.42*	*1.03*
Dimethylcyclopentanedione (126) (or isomer)	126	0.00	tr	0.01	0.02	0.10
Dimethylcyclopentanedione (126) (or isomer)	126	0.00	0.00	0.01	0.02	0.07
Dimethyloctadienone (152) (or isomer)	95	0.00	0.07	0.19	0.38	0.86
Alkanals		*2.80*	*43.12*	*53.74*	*50.14*	*75.88*
Butanal (72)*	72	0.00	0.05	0.11	0.11	0.16
Pentanal (86)*	44	0.60	5.25	5.94	6.23	11.35
Hexanal (100)*	56	1.86	34.95	43.45	39.76	55.69
Heptanal (114)*	70	0.07	0.80	0.96	0.64	1.44
Octanal (128)*	43	0.00	1.02	1.87	1.89	3.28
Nonanal (142)*	57	0.24	0.95	1.24	1.26	3.07
Decanal (156)	57	0.03	0.10	0.17	0.25	0.89
Alkenals		*0.63*	*11.90*	*58.76*	*72.72*	*99.10*
(*E*)-2-Propenal (56)	56	0.22	0.25	0.32	0.51	0.58
(*E*)-2-Butenal (70)	70	0.00	0.00	0.28	0.34	0.27
(*E*)-2-Pentenal (84)	55	0.00	0.07	0.33	0.34	0.52
(*E*)-2-Hexenal (98)*	41	0.00	0.40	2.31	2.81	3.84
Heptenal (112) (or isomer)	83	0.00	0.10	0.67	0.67	1.03
(*E*)-2-Heptenal (112)*	83	0.41	5.32	19.40	22.77	26.94
Octenal (126) (or isomer)	83	0.00	0.68	5.30	9.89	10.90
(*E*)-2-Octenal (126)*	41	0.00	4.47	26.83	27.80	42.02
Nonenal (140) (or isomer)	83	0.00	0.05	0.40	0.71	1.43
(*E*)-2-Nonenal (140)*	41	0.00	0.18	1.03	3.23	4.67
(*E*)-2-Decenal (154)	70	0.00	0.28	1.35	2.57	4.66
(*E*)-2-Undecenal (168)	70	0.00	0.10	0.54	1.08	2.24
Di- and Trialkenals		*0.00*	*2.46*	*20.06*	*71.93*	*65.56*
2,4-Heptadienal (110)	110	0.00	0.00	0.21	0.51	0.47
(*Z,E*)-2,4-Octadienal (124)	81	0.00	tr	0.28	0.29	0.36
(*E,E*)-2,4-Octadienal (124)	81	0.00	0.10	1.02	1.41	1.63
(*Z,E*)-2,4-Nonadienal (138)	81	0.00	0.10	0.68	1.76	2.15
(*E,E*)-2,4-Nonadienal (138)*	81	0.00	0.31	2.82	8.09	8.18
(*Z,E*)-2,4-Decadienal (152)*	81	0.00	0.98	5.47	20.07	14.35
(*E,E*)-2,4-Decadienal (152)*	81	0.00	0.97	9.54	39.50	37.98
Decatrienal (150) (or isomer)	79	0.00	0.00	0.00	0.07	0.08
Decatrienal (150) (or isomer)	79	0.00	0.00	0.04	0.14	0.14
(*Z,E*)-2,4-Undecadienal (166) (or isomer)	81	0.00	0.00	tr	0.04	0.10
(*E,E*)-2,4-Undecadienal (166)	81	0.00	0.00	0.00	0.05	0.12
Aromatic aldehydes and ketones		*0.00*	*0.19*	*0.44*	*1.67*	*2.41*
Benzaldehyde (106)*	106	0.00	0.14	0.29	1.27	1.83
1-Phenylethanone (120)	105	0.00	0.02	0.05	0.21	0.25
Methylbenzaldehyde (120)	119	0.00	0.00	0.02	0.02	0.03
Ethylbenzaldehyde (134)	134	0.00	0.02	0.05	0.09	0.15
Ethylbenzaldehyde (134) (isomer)	134	0.00	0.01	0.03	0.05	0.11
4-(1-Methylethyl)-benzaldehyde (148)	133	0.00	0.00	0.00	0.03	0.04

[a] Asterisked compounds were acquired commercially and used as standards for identification purposes; tr traces.

Table 5
Oxygenated aldehydes, lactones, furan derivatives and phenol derivatives detected in the headspace of S6, S15, S17, S18 and S22 sunflower oil samples and their abundances expressed as area counts of their mass spectra base peak (Bp) divided by 10^6, obtained as average of two determinations

Compound (molecular weight)[a]	Bp	S6	S15	S17	S18	S22
Oxygenated aldehydes		*0.00*	*1.96*	*7.60*	*14.19*	*22.16*
2,3-Epoxyhexanal (114) (or isomer)	71	0.00	1.17	2.25	2.24	3.51
2,3-Epoxyoctanal (142) (or isomer)	71	0.00	0.63	1.89	1.46	2.43
4-Oxo-(*E*)-2-octenal (140) (or isomer)	55	0.00	0.00	0.09	0.14	0.17
4-Oxooctanal (142)	85	0.00	0.00	0.25	0.23	0.57
4-Hydroxy-(*E*)-2-heptenal (128) (or isomer)	57	0.00	0.00	0.00	tr	0.26
4-Oxo-(*E*)-2-nonenal (154)*	55	0.00	0.07	0.13	0.33	0.73
4-Oxononanal (156)	43	0.00	0.00	0.26	0.45	1.60
4-Hydroxy-(*E*)-2-octenal (142) (or isomer)	57	0.00	0.00	1.10	2.63	3.65
4-Oxononenal (154) (or isomer)	55	0.00	0.00	0.00	0.22	0.29
4,5-Epoxy-2-nonenal (154)	68	0.00	0.00	0.05	0.16	0.17
2,3-Epoxydecanal (170) (or isomer)	71	0.00	tr	0.07	0.13	0.21
4,5-Epoxy-2-nonenal (154) (or isomer)	68	0.00	0.00	0.12	0.55	0.51
4-Hydroxy-(*E*)-2-nonenal (156)*	57	0.00	0.00	0.60	3.14	6.28
4,5-Epoxy-2-decenal (168) (or isomer)	68	0.00	0.02	0.20	0.58	0.47
4,5-Epoxy-2-decenal (168)*	68	0.00	0.07	0.59	1.93	1.31
Lactones		*0.00*	*0.45*	*3.10*	*8.62*	*20.73*
5-Ethyl-dihydro-2(3h)-furanone (114)	85	0.00	0.24	0.53	1.61	3.27
5-Propyl-dihydro-2-(3h)-furanone (128)	85	0.00	0.02	0.04	0.19	0.40
5-Butyl-2(3h)-furanone (140)	98	0.00	0.00	0.10	0.17	0.42
5-Butyl-2(5h)-furanone (140)	84	0.00	0.08	0.48	1.45	3.01
5-Butyl-dihydro-2(3h)-furanone (142)	85	0.00	0.00	0.18	0.53	1.18
5-Pentyl-2(3h)-furanone (154)	98	0.00	0.03	0.25	0.56	1.30
5-Pentyl-2(5h)-furanone (154)	84	0.00	0.02	1.05	2.77	6.66
5-Pentyl-dihydro-2(3h)-furanone (156)	85	0.00	0.06	0.27	0.67	1.85
Delta-deca-2,4-dienolactone (166)	95	0.00	0.00	0.02	0.03	0.05
5-Hexyl-dihydro-2(3h)-furanone (170)	85	0.00	0.00	0.00	0.03	0.05
5-Hydroxy-2-decenoic acid lactone (168)	97	0.00	0.00	0.18	0.61	2.54
Furan derivatives		*0.23*	*8.32*	*35.41*	*39.75*	*77.89*
2-Propyl-furan (110)	81	0.00	0.00	0.00	0.01	0.02
2-Butyl-furan (124)	81	0.00	0.80	2.80	3.46	8.39
2-Butyl-furan isomer (124)	81	0.00	0.00	0.05	0.07	0.09
2-Pentyl-furan (138)*	81	0.23	7.35	32.23	35.50	68.00
2-Pentyl-furan (138) (isomer)	81	0.00	0.02	0.09	0.18	0.38
2-Pentyl-furan (138) (isomer)	81	0.00	0.15	0.23	0.48	0.88
2-Hexyl-furan (152)	152	0.00	0.00	0.00	0.02	0.06
2-Heptyl-furan (166)	166	0.00	0.00	0.01	0.02	0.05
Trimethylbenzofuran (160) (or isomer)	160	0.00	0.00	0.00	0.01	0.02
Phenol derivatives		*0.00*	*0.03*	*0.23*	*0.32*	*0.48*
Phenol (94)*	94	0.00	0.00	0.02	0.12	0.20
2-Propylphenol (136) (or isomer)	107	0.00	0.02	0.13	0.15	0.21
3-(1-Methylethyl)-phenol (136) (or isomer)	121	0.00	0.01	0.08	0.05	0.07

[a] Asterisked compounds were acquired commercially and used as standards for identification purposes; tr traces.

four to ten carbon atoms were detected in the headspace of the non-oxidized samples and their concentration increases as the oxidation degree of the samples does; among these aldehydes, hexanal is one of the main, followed by heptanal, and pentanal. Although only some alkenals were present in the headspace of non-oxidized samples, a numerous group of compounds of this nature has been found in oxidized samples, mainly 2-alkenals, being 2-octenal and 2-heptenal the ones whose concentration increases more with the oxidation level of the sample.

Alkadienals were not detected in the headspace of the non-oxidized samples but they appear with the highest proportions in the most oxidized samples (see Table 4); the alkadienals detected have from 7 to 11 carbon atoms, and the most important are those having nine and 10 carbon atoms. It is noteworthy that in the headspace of the samples which are at the first oxidation stages, such as S15, the area counts of (*Z*,*E*)-2,4-decadienal is higher than that of (*E*,*E*)-2,4-decadienal, and that in samples in a more advanced oxidation state this proportion is reversed, especially in the most oxidized samples S18 and S22. These results are supported by those obtained in the study of the oil liquid phase of these same samples using ^{1}H NMR, which showed a higher proportion of hydroperoxides supporting (*Z*,*E*)-unsaturated dienic systems than (*E*,*E*)-unsaturated dienic systems, specially in samples being in early oxidation stages (Guillén & Goicoechea, 2007).

In addition, as Table 4 shows, two isomers of decatrienal have been also detected in the headspace of oxidized sunflower oil samples; the tentative identification of these compounds has been made on the basis of its mass spectra by comparison with that of some nonatrienals (Schuh & Schieberle, 2005).

Aromatic aldehydes, such as benzaldehyde and some of its alkyl derivatives, and aromatic ketones, such as phenyl ethanone, were absent in the non-oxidized oil samples, such as S6 (see Table 4), and their concentrations increased with the oxidation degree of the samples.

As Table 5 shows, the headspace of oxidized sunflower oil samples, in addition to the aldehydes above mentioned, contains others having one additional oxygenated functional group. Among those detected there are some tentatively identified as 2,3-epoxy-alkanals, such as 2,3-epoxyhexanal, 2,3-epoxyoctanal and 2,3-epoxydecanal, being 2,3-epoxyhexanal the main one (Buettner & Schieberle, 2001a). Oxo-alkanals, such as 4-oxooctanal and 4-oxononanal, have been detected too; other authors have also found some oxo-alkanals in oils submitted to frying conditions (Takeoka, Buttery, & Perrino, 1995, 1996). In addition to oxygenated saturated aldehydes, a significant number of oxygenated α,β-unsaturated aldehydes, named as OαβUAs (Guillén & Goicoechea, 2008), have also been detected. Among them 4-hydroxy-(*E*)-2-alkenals, 4-oxo-(*E*)-2-alkenals and 4,5-epoxy-2-alkenals have been detected, mainly 4-hydroxy-(*E*)-2-nonenal, 4-oxo-(*E*)-2-nonenal and 4,5-epoxy-2-decenals.

It should be pointed out that 4-oxo-(*E*)-2-nonenal was detected in samples being at earlier oxidation stages (sample S15) and 4-hydroxy-(*E*)-2-nonenal was detected in samples more oxidized (sample S17). By contrast, the area counts of this latter compound in samples having a high oxidation level is nearly nine times higher than that of the former (see Table 5, S22). Although 4-oxo-(*E*)-2-alkenals have been detected in the headspace of these oxidized oil samples, in the liquid phase of the same samples studied by ^{1}H NMR they were not detected (Guillén & Goicoechea, 2007). By contrast, using this second technique, 4-hydroperoxy-(*E*)-2-alkenals were detected in the liquid phase of these samples, though they have not been detected in their headspace. The absence of 4-oxo-(*E*)-2-alkenals in the oil liquid phase and their detection in the headspace of the same samples, and the absence of 4-hydroperoxy-(*E*)-2-alkenals in the headspace and their presence in the liquid phase, suggest the possibility of 4-hydroperoxy-(*E*)-2-alkenals, which are very unstable and reactive compounds, being degraded in the chromatographic run into 4-oxo-(*E*)-2-alkenals; this is in agreement with the results obtained by other authors (Gardner & Grove, 1998). The simultaneous identification and determination of this group of OαβUAs in oxidized oils is noteworthy because most of the studies on the occurrence of compounds of this nature in foods refer to only one or two OαβUAs (Guillén & Goicoechea, 2008).

The importance of these findings is due to the genotoxicity and cytotoxicity of these compounds. Thus, 4-hydroxy-(*E*)-2-nonenal, is also produced in cells and tissues in the oxidation of polyunsaturated fatty acids and esters, and has been considered as potential causal agent of several degenerative diseases, like Alzheimer's, Parkinson's, cancer, etc (Esterbauer et al., 1991; Zarkovic, 2003). Recent studies have shown that other oxygenated α,β-unsaturated aldehydes detected in this study, such as 4-oxo-(*E*)-2-nonenal or 4,5-epoxy-(*E*)-2-decenal and homologues, have similar reactivity to 4-hydroxy-(*E*)-2-nonenal (Jian, Arora, Oe, Shuvaev, & Blair, 2005). Furthermore, taking into account that most of these compounds are formed simultaneously in the oxidation process, it could be thought that the same can happen in cells and tissues, although the attention has been focused almost exclusively on 4-hydroxy-(*E*)-2-nonenal. It should be pointed out that 4,5-epoxy-2-decenal has also been considered to be potential causal agent of cardiovascular diseases (Jian et al., 2005); however, it is considered important in the flavour of different foods, such as grapefruit juice (Buettner & Schieberle, 2001b), and it is included in the EAFUS list of substances ("Everything Added to Food in the United States"), which contains ingredients added directly to food that FDA (US Food and Drug Administration) has either approved as food additives or listed or affirmed as GRAS (Generally Recognized As Safe).

In addition to these compounds, others very closely related have also been found in the headspace of oxidized samples. Among them there are five dihydro-5-alkyl-2(3H)-furanones from 6 to 10 carbon atoms; some of these furanones were previously detected among the volatile constituents of used frying oils (Takeoka et al., 1996). And finally, two 5-alkyl-2(3H)-furanones and two 5-alkyl-2(5H)-furanones having eight and nine carbon atoms have been detected, as Table 5 shows. It should be pointed out that 5-pentyl-2(5H)-furanone was identified by the mass spectra data given by Bonete and Najera (Bonete & Najera, 1994); this compound was previously detected among the odorants of French fries fried in palm oil (Wagner & Grosch, 1997).

As can be observed in Table 5, some furan alkyl derivatives have also been detected in the headspace of the samples subject of study; among these compounds the main ones are pentyl- and butyl-furan, the first detected even in the non-oxidized S6 sample; the other ones are only present in samples with a certain oxidation degree.

In conclusion, in the oxidation of sunflower oil taking place at room temperature in closed receptacles, and as a consequence in the presence of limited amounts of oxygen, the well known oxidation compounds, such as acids, alcohols, esters, hydrocarbons, ketones, alkanals, alkenals and alkadienals are formed. It has also been observed, for the first time, that oils oxidized under these conditions contain certain monoaromatic and polyaromatic hydrocarbons, these latter of low molecular weight from naphthalene to fluorene, with concentrations that rise in line with the oxidation level reached by the samples; this fact suggests that the oxidation at room temperature in presence of limited amounts of air provokes the formation of these compounds and this fact has been proved in a parallel study (Guillén et al., 2008). The occurrence of aromatization reactions is also evidenced by the significant presence of some aromatic aldehydes and ketones.

In addition, it has been shown for the first time the formation of oxygenated derivatives of alkanals, as well as of some toxic oxygenated α,β-unsaturated aldehydes having hydroxy, oxo, and epoxy functional groups in the oxidation of sunflower oil at room temperature in closed containers. It should be pointed out that although attention on compounds of this nature has been focussed mainly on 4-hydroxy-(*E*)-2-nonenal until now, all these compounds are produced simultaneously and show similar biological activity (Guillén & Goicoechea, 2008). Taking into account that 4-hydroxy-(*E*)-2-nonenal has been proved to form in cells and tissues as consequence of oxidative stress processes affecting

omega-6 acyl groups, and that this compound, together with many other toxic oxygenated α,β-unsaturated aldehydes, are formed in the oxidation at room temperature of the sunflower oil samples here studied, the possibility of the formation in cells and tissues of many of the toxic compounds detected here should be considered. As said before, among these compounds there are, in addition to several oxygenated α,β-unsaturated aldehydes, also mono and polycyclic aromatic hydrocarbons which could explain the presence of compounds of this latter nature in tissues of animal or plants (Wilcke, Amelung, Martius, Garcia, & Zech, 2000) growing in uncontaminated environments.

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